Study of atrophy in Facioscapulohumeral muscular dystrophy (FSHD)

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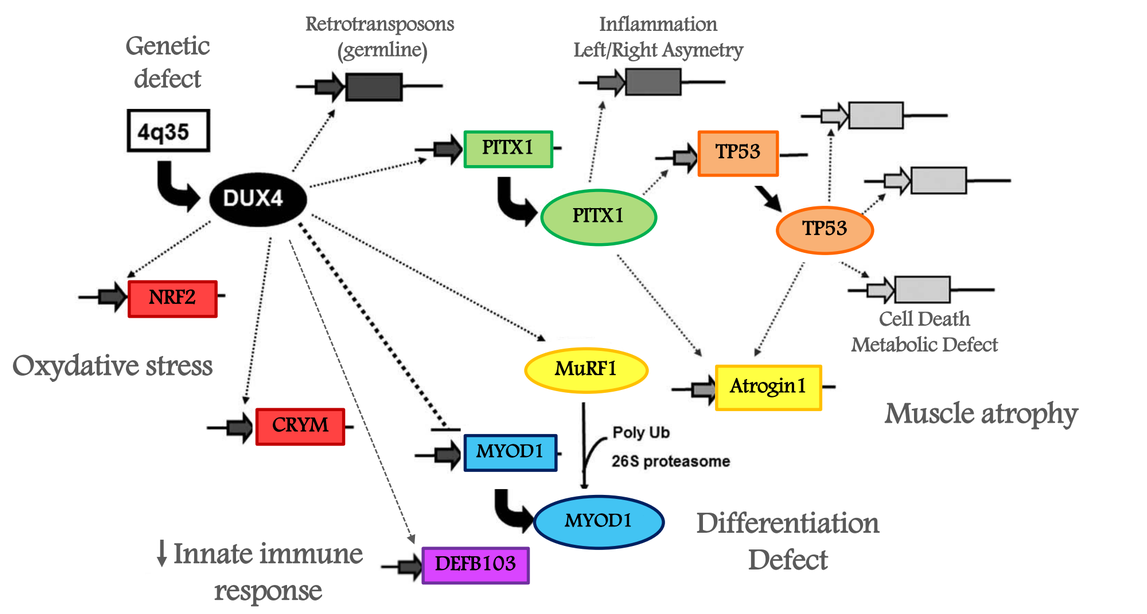
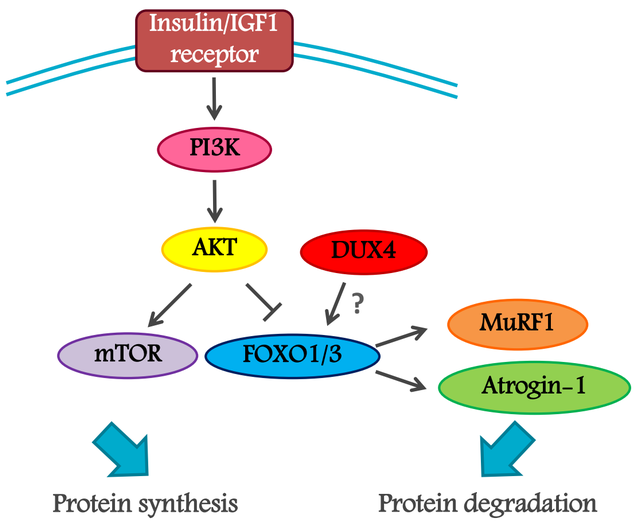
Cascade of gene deregulation in FSHD



Vanderplanck et *al.*, 2011

Atrophy/hypertrophy balance



FSHD muscle cells : ↗ DUX4

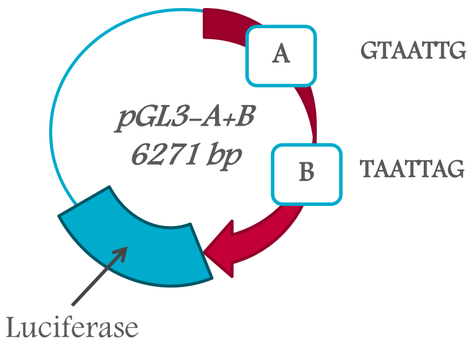
↗ MuRF1 and Atrogin-1

FOXO1 = transcription activator of the atrogenes

Two putative DUX4 binding sites in the *FOXO1* promoter

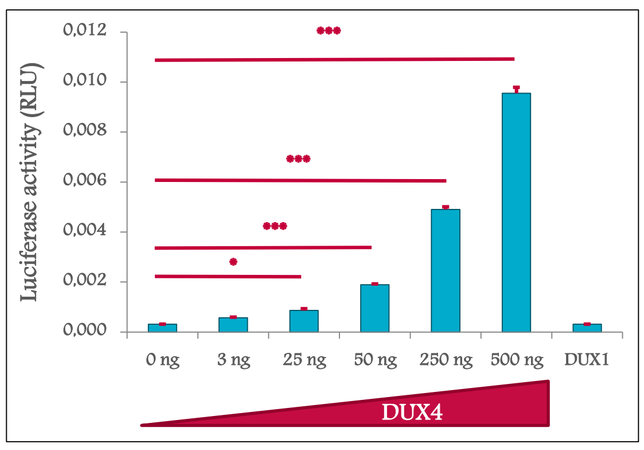
Aim : to investigate whether the atrogene expression we observe in FSHD muscle cells is dependent on FOXO1 transactivation by DUX4.

Construction of reporter vectors



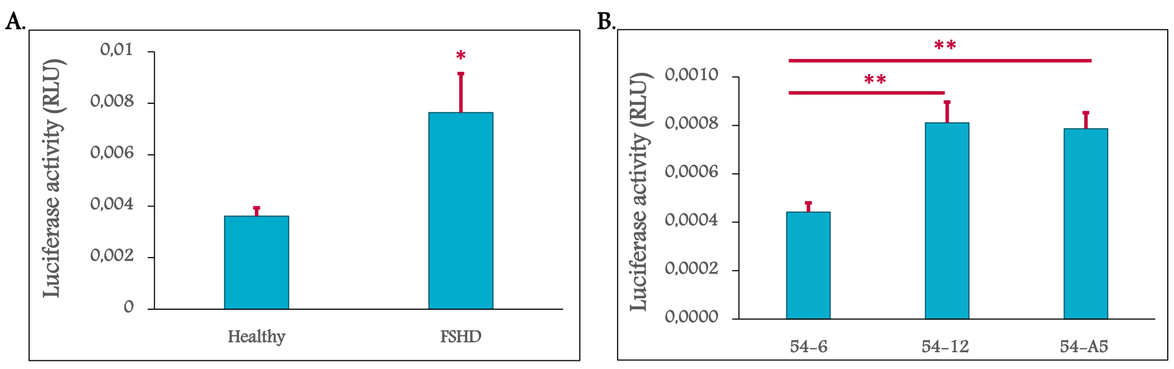
Reporter vector containing the luciferase gene under the control of the *FOXO1* promoter with two (*pGL3-A+B ;1453 bp*) putative DUX4 binding sites.

Effect of DUX4 on the *FOXO1* promoter



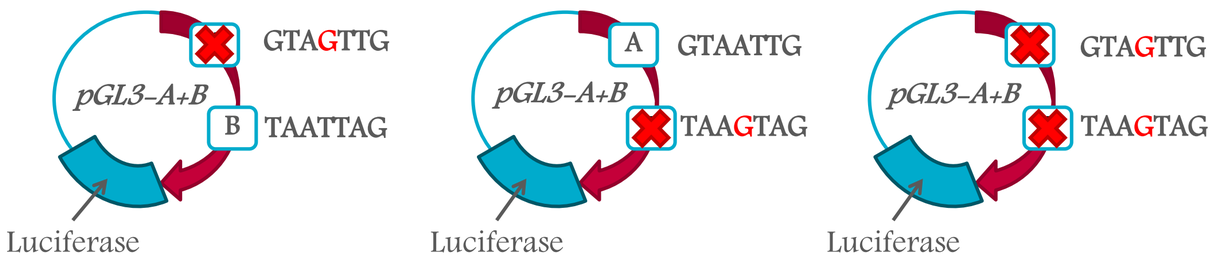
Activation of the *FOXO1* promoter by the DUX4 protein. C2C12 myoblasts were co-transfected with 500 ng of the luciferase reporter vector containing two DUX4 putative binding sites, and with *pCIneo-DUX4* or *pCIneo-DUX1*. Cells were harvested 24 hours later and lysates were used for luciferase activity assay. The significance of the differences between experiments was evaluated with Anova test.* : p< 0,05 ; *** : p< 0,001.

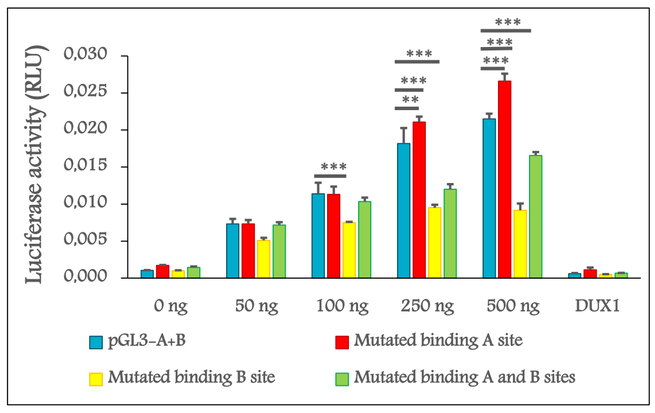
Activation of the *FOXO1* promoter in FSHD myoblasts



Activity of the *FOXO1* promoter containing two (A+B) DUX4 putative binding sites. Either healthy or FSHD immortalized myoblasts (A) and healthy (54-6) or FSHD (54-12 and 54-A5) immortalized "mosaic" myoblasts (B) were transfected with 500 ng of *pGL3-A+B* and with the Renilla luciferase (control vector). Cells were harvested 24 hours later and lysates were used for luciferase assay. Each value corresponds to biological triplicate. The error bar represents the standard deviation calculated from the ratio between firefly and Renilla luciferase luminescence (internal control). The significance of the differences between experiments was evaluated with Student's t-test.* : p< 0,05 ; ** : p< 0,01.

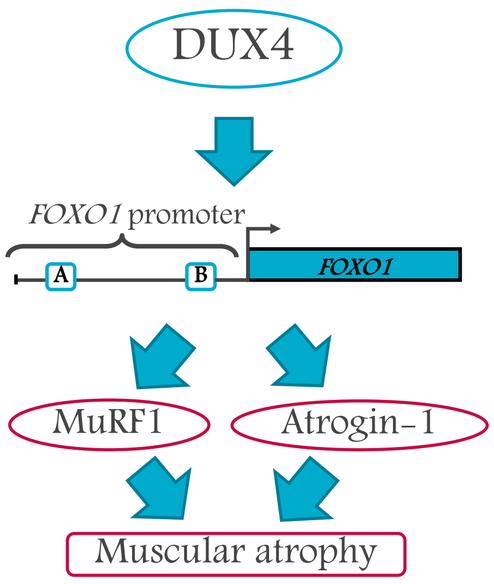
Site-directed mutagenesis of DUX4 putative binding sites





Mutation of the DUX4 binding B site decreases the activation of the *FOXO1* promoter by DUX4. C2C12 myoblasts were co-transfected with 500 ng WT (blue) or mutated *pGL3-A+B* (red, yellow and green) and with *pCIneo-DUX4* or *pCIneo-DUX1*. Cells were harvested 24 hours later and lysates were used for luciferase assay. The significance of the differences between experiments was evaluated with Anova test , ** : p< 0,01 ; *** : p< 0,001.

Conclusion and perspectives



The *FOXO1* promoter is activated in FSHD myoblasts probably due to the DUX4 induction in these cells. The *FOXO1* promoter is activated by DUX4 in a dose-dependent manner and we can observe a decrease in the luciferase activity following mutation of the DUX4 binding B site. However, DUX4 even following the mutation of the DUX4 binding sites, DUX4 is still able to activate the *FOXO1* promoter activity independently of direct DUX4 binding. To confirm DUX4 binding to the *FOXO1* promoter, we will perform chromatin immunoprecipitation.

FOXO1 could therefore constitute a novel DUX4 target and could be a direct link between DUX4 expression and the development of atrophy in FSHD.

Acknowledgements

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